Response to Horn *et al.*

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TO THE EDITOR

We thank Horn *et al.* (2012) for mentioning the Genetic Alliance, the Pseudoxanthoma Elasticum International Registry, and the National Psoriasis Foundation and providing Internet links to these registries in their letter regarding our commentary ''Significance of Patient Registries for Dermatological Disorders'' (de Souza and Miller, 2012). Table 1 in our commentary included a select number of registries and was not meant to be an exhaustive or all-inclusive list.

With regard to the comment from Horn *et al.* (2012) that Table 1 erroneously lists industry funding for the Hereditary Angioedema Association (HAEA) registry, we listed the HAEA as the sponsor of the HAEA registry and noted that the HAEA receives funding from the industry sponsors listed in Table 1, as the HAEA lists these sponsors on their registry's webpage.

The Cystic Fibrosis (CF) Registry was mentioned as a gold standard because, as stated in the commentary, it has enrolled a large percentage (88%) of the estimated number of CF patients in the United States. The data from the CF Registry are easily accessible in annual reports published by the CF Foundation. It would be helpful if similar annual reports for the registries listed in Table 1 are made publicly available by their sponsors. This will facilitate the comparisons between the registries suggested by Horn *et al.*

We thank Horn *et al.* (2012) at the Genetic Alliance, the National Psoriasis Foundation, and the Pachyonychia Congenita Project for their valuable contributions to patient advocacy and registries for dermatological and other disorders.

Abbreviations: CF, cystic fibrosis; HAEA, Hereditary Angioedema Association

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Elevated Matrix Metalloproteinases and Collagen Fragmentation in Photodamaged Human Skin: Impact of Altered Extracellular Matrix Microenvironment on Dermal Fibroblast Function

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TO THE EDITOR

Exposure of the human skin to acute solar UV irradiation induces several members of the matrix metalloproteinase family (MMPs), which degrade collagen fibrils and other components of the dermal extracellular matrix (ECM) (Fisher *et al.*, 1996; Yaar and Gilchrest, 2007). Elevation of MMPs due to chronic sun exposure can promote accumulation of dermal ECM fragmentation, which contributes to impaired skin function and aged appearance of chronically sun-exposed skin (Fisher *et al.*, 1997). Histological and ultrastructural studies have revealed that there are major alterations in dermal ECM, most notably the accumulation of amorphous elastin-containing material in the upper dermis, and disorganized collagen fibrils, which constitute the bulk (90% dry weight) of skin connective tissue.

Elevation of MMPs and consequent dermal collagen fibril fragmentation following acute UV irradiation in human skin is well characterized (Fisher *et al.*, 1996; Quan *et al.*, 2009). However, the expression of MMPs in clinically photodamaged human skin and the role

Abbreviations: ECM, extracellular matrix; MMPs, matrix metalloproteinase family; 3D, three dimensional; TIMP, tissue inhibitor of metalloproteinases

of fragmented collagen microenvironment in cellular function have not been extensively studied. Here we quantified the gene expression of all 23 known mammalian MMPs (Egeblad and Werb, 2002) in the photodamaged forearm and subject-matched sun-protected underarm skin, from 19 individuals, and the effect of collagen fragmentation on dermal fibroblast function, the major cells responsible for collagen homeostasis in skin. All procedures involving human subjects were approved by the University of Michigan Institutional Review Board, in adherence to the Helsinki guidelines, and provided written informed consent before entering the study.

The relative expression levels of MMPs in underarm skin are shown in Figure 1a. Transcripts for MMP-8, -10, -13, -20, and -26 were not detected. MMP-14, -2, -3, -28, -7, and -15 were the most highly expressed, while the remaining MMPs were expressed at lower levels. Among the 18 MMPs expressed in human skin, 7 were significantly elevated in photodamaged forearm, compared with sun-protected underarm skin (fold-increase from high to low: MMP-9, 5.3-fold; MMP-27, 5.1-fold; MMP-3, 3.0-fold; MMP-11, 2.7-fold; MMP-17, 2.2-fold; MMP-1, 1.9-fold; and MMP-2, 1.6-fold) (Figure 1b). To quantify the relative contributions to elevated MMPs, epidermis and dermis were separated by laser capture microdissection. Figure 1c shows that all MMPs that are elevated in photodamaged skin, except MMP-3, were primarily expressed in the dermis (MMP-27, 86%; MMP-9, 85%; MMP-2, 83%; MMP-11, 82%; MMP-17, 62%; and MMP-1, 59%).

MMPs and tissue inhibitor of metalloproteinases (TIMPs) are often coordinately regulated as a means to control excess MMP activity; we also investigated whether TIMPs are elevated in photodamaged skin. We found that all four known TIMP genes (*TIMP-1, -2, -3,* and *-4*) are primarily expressed in human skin dermis; however, no differences in mRNA levels of TIMP-1, -2, -3, or -4 were found between sun-protected underarm and sun-exposed forearm skin (Figure 1d).

The observed preferential induction of MMPs relative to TIMPs suggests that MMP activities are elevated in photodamaged skin. To access MMP activity, we performed *in situ* zymography, in which unfixed skin sections are placed over a layer of fluorescently labeled collagen. As shown in Figure 1e, elevated MMP activity in photodamaged skin resulted in breakdown of the collagen, resulting in loss of fluorescence. In addition, production of type-I collagen, the major structural protein in skin, was significantly reduced in laser capture microdissection-captured photodamaged forearm dermis, compared with sun-protected underarm dermis (Figure 1f and g). These data indicate aberrant collagen homeostasis, i.e., increased multiple MMPs and reduced collagen production, in photodamaged dermis.

Given that dermal fibroblasts are primarily responsible for collagen production and turnover *in vivo,* data presented above indicate that impaired dermal fibroblast function contributes to aberrant collagen homeostasis in photodamaged skin. We have previously reported that, in standard monolayer culture, collagen and MMP-1 expression in fibroblasts from photodamaged and sun-protected skin are similar (Varani *et al.*, 2001), suggesting that the fragmented extracellular microenvironment within photodamaged dermis may trigger abnormal fibroblast function.

To examine this possibility, we cultured dermal fibroblasts in intact or fragmented three-dimensional (3D) collagen lattices to model sun-protected and sun-exposed dermis, respectively. Collagen lattices were fragmented by controlled exposure to purified human MMP-1 (Fisher *et al.*, 2009). Atomic force microscopy indicated that intact (Figure 2a, upper left panel) and MMP-1-fragmented (Figure 2a, upper right panel) collagen lattices resemble collagen fibrils in sun-protected underarm (Figure 2a, lower left panel) and sun-exposed forearm (Figure 2a, lower right panel), respectively. Measurement of all 23 known mammalian MMPs indicated that transcripts for MMP-13, MMP-20, and MMP-26, which are undetectable in human skin *in vivo,* and non-fibroblast cell-type-specific MMPs (MMP-8, neutrophil collagenase; MMP-9, 92 kDa gelatinase-B; MMP-28, epilysin) were not detected in human dermal fibroblasts in 3D collagen lattices. Interestingly, all MMPs that were found to be elevated in photodamaged dermis *in vivo*, except MMP-17, were elevated in fibroblasts cultured in fragmented 3D collagen lattices (fold-increase from high to low: MMP-1, 4.5-fold; MMP-27, 3.2-fold; MMP-11, 3.1-fold; MMP-2, 2.4-fold; and MMP-3, 1.9-fold) (Figure 2b). Consistent with the above *in vivo* observations, no differences in mRNA levels of TIMP-1, -2, -3, or -4 were found between cells cultured in garmented versus intact 3D collagen lattices (Figure 2c). Type-I collagen mRNA (Figure 2d) and protein (Figure 2e) levels were significantly reduced in fibroblasts cultured in fragmented 3D collagen lattices, as is observed in photodamaged forearm dermis. Thus, collagen fragmentation recreates many of the abnormalities seen in photodamage *in vivo*. These data indicate that fragmentation of the collagenous ECM in photodamaged dermis alters collagen homeostasis by influencing the function of dermal fibroblasts.

MMPs comprise a large family of proteinases that are capable of degrading every type of dermal ECM protein. Studies conducted by us and others over the past several years have shown that acute UV irradiation transiently induces expression of only three MMPs in resident human skin cells *in vivo*, i.e., interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), and 92 kDa gelatinase (MMP-9) (Fisher *et al.*, 1996; Brenneisen *et al.*, 2002; Quan *et al.*, 2009). Our data indicate that, compared with acute UV irradiation, a larger variety of MMPs, including UV-inducible MMPs, are constitutively elevated in photodamaged skin. Interestingly, compared with acute UV irradiation, in which the epidermis is the major source of transiently induced MMPs (Quan *et al.*, 2009), the dermis is the major source of elevated MMPs in photodamaged skin. Elevated MMPs in photodamaged dermis can be divided into following groups: collagenases, MMP-1; gelatinases, MMP-2; stromelysins, MMP-3, MMP-9, and MMP-11; membrane-associated, MMP-17 and the recently identified MMP-27. In addition, compared with acute UV irradiation, in which TIMP-1 is significantly induced

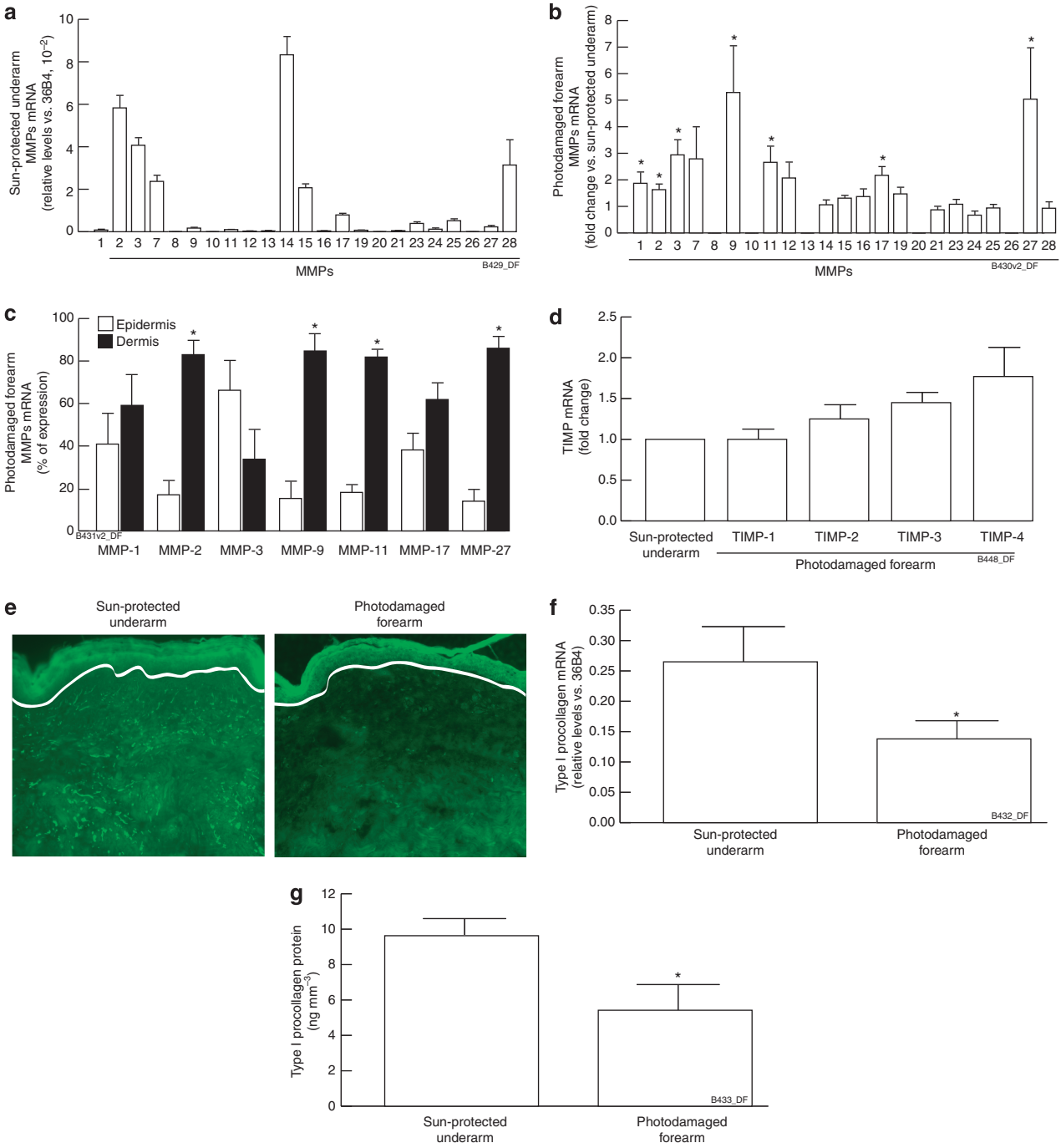

Figure 1. Elevated expression of multiple matrix metalloproteinase family (MMPs) and reduced production of type-I procollagen in photodamaged forearm human dermis. (**a**) Basal gene expression of MMP family members in human underarm skin. $N=19$. (**b**) Multiple MMPs elevated in photodamaged forearm skin relative to sun-protected underarm skin. $N=19$, $^*P<0.05$. (**c**) Elevated MMPs in the dermis of photodamaged forearm skin. $N=6$, $^*P<0.05$. (**d**) Similar tissue inhibitor of metalloproteinase *(TIMP)* gene expression in sun-protected and photodamaged skin, $N=10$. (**e**) Elevated collagenase activity in the dermis of photodamaged forearm skin determined by *in situ* zymography. Loss of green fluorescence in photodamaged dermis indicates degradation of fluorescein-collagen substrate. White lines indicate the boundary between the epidermis (top) and dermis (bottom). $N=6$. (**f**) Reduced type-I procollagen gene expression in photodamaged forearm dermis. $N=19$, $^*P<0.05$. (**g**) Reduced type-I procollagen protein levels in photodamaged forearm dermis. $N=19$, $^*P<0.05$. (**c, f, g**) Dermis was isolated by laser capture microdissection. All results are means ± SEM.

(Fisher *et al.*, 1997), elevated MMPs in photodamaged skin are not accompanied by alterations of TIMP expression. It is tempting to speculate that the combined actions of the wide variety of MMPs that are constitutively elevated in photodamaged dermis are involved in progressive degradation of dermal ECM.

Dermal fibroblasts are the primary cells that are responsible for collagen production and turnover in skin. Our data support the concept that fragmentation

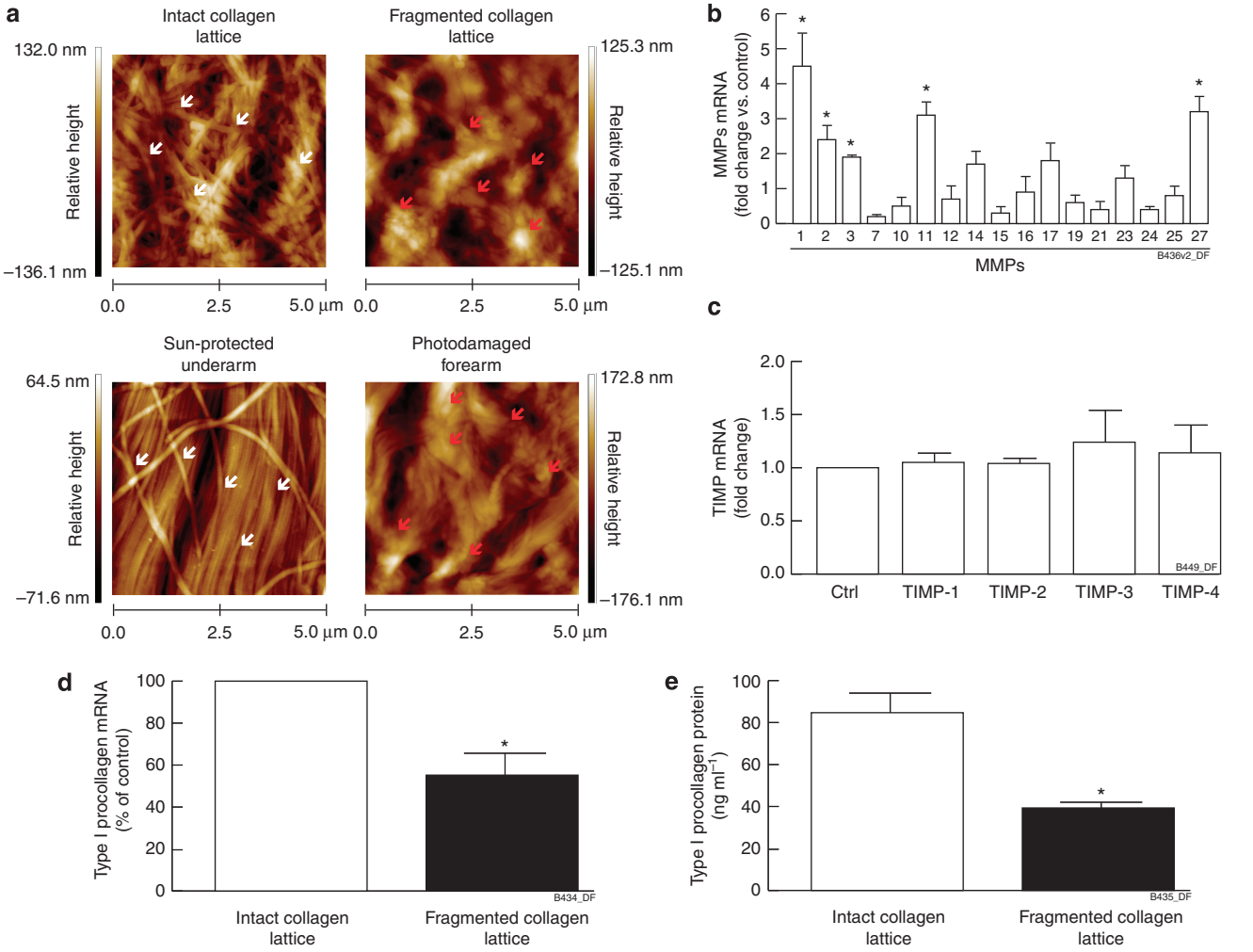

Figure 2. Collagen fibril fragmentation alters dermal fibroblast collagen homeostasis. (**a**) Nanoscale collagen fibrils were imaged by atomic force microscopy. The white and red arrows indicate intact and fragmented/disorganized collagen fibrils, respectively. Images are representative of six independent experiments. (**b**) Matrix metalloproteinases family (MMPs) that are elevated in photodamaged dermis are induced in fibroblasts cultured in MMP-1-fragmented collagen lattices. $N=3$, $^*P<0.05$. (**c**) Tissue inhibitor of metalloproteinase *(TIMP)* gene expression is not elevated in fibroblasts cultured in MMP-1-fragmented collagen lattices. $N=4$. (**d**) Type-I procollagen mRNA levels are reduced in the fibroblasts cultured in MMP-1-fragmented collagen lattices. $N=9$, $^*P<0.05$. (**e**) Type-I procollagen protein levels are reduced in the fibroblasts cultured in MMP-1-fragmented collagen lattices. $N=5$, $^*P<0.05$. All results are means ± SEM.

of the dermal collagenous ECM alters dermal fibroblast function to shift the balance to produce more MMPs and less collagen in photodamaged skin. A wealth of evidence indicates that tissue microenvironment controls a variety of cellular processes including signal transduction, gene expression, and tissue homeostasis (Varani *et al.*, 2004; Spencer *et al.*, 2007; Fisher *et al.*, 2009; Bissell and Hines, 2011). One important finding of our study is that alterations of the dermal ECM microenvironment brought about by chronic exposure to solar UV irradiation have significant consequences on the regulation of collagen homeostasis by fibroblasts. Currently, mechanisms by which ECM microenvironment in photodamaged human skin control fibroblast function are not well understood. We previously reported that fragmented collagen is unable to support normal cell shape and mechanical tension within fibroblasts, and this loss of cell shape and mechanical tension is closely associated with increased transcription factor AP-1 (Fisher *et al.*, 2009). Given that AP-1 functions as a major driving force for multiple MMPs and potent negative regulator of type-I procollagen expression, it is conceivable that AP-1 activity induced by fragmented collagen microenvironment significantly contributes to elevated MMPs and loss of type-I collagen expression in photodamaged skin. In addition, fragmented collagen in photodamaged skin may also impair mechanical properties of the dermal collagen network, collagen–integrin mechanical sensing, and subsequent integrin signaling events associated with aberrant collagen homeostasis.

In summary, photodamaged dermis constitutively expresses elevated levels of several MMPs and reduced production of type-I collagen, which likely lead to chronic, progressive degradation of the dermal collagenous ECM and loss of collagen in photodamaged human skin. This aberrant collagen homeostasis largely results from altered fibroblast

function due to long-term consequences of fragmented dermal collagen microenvironment in photodamaged human skin.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Rare Pathogenic Variants in *IL36RN* Underlie a Spectrum of Psoriasis-Associated Pustular Phenotypes

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TO THE EDITOR

Mutations of the *IL36RN* gene have been recently identified in patients affected by generalized pustular psoriasis (GPP), a neutrophilic dermatosis that presents as an acute pustular eruption accompanied by features of systemic inflammation (Marrakchi *et al.*, 2011; Onoufriadis *et al.*, 2011). Although GPP is traditionally classified as a variant of psoriasis vulgaris (PV; Griffiths and Barker, 2010), our group has demonstrated that the two conditions are genetically distinct (Onoufriadis *et al.*, 2011).

In this context, the aim of this study was to characterize the spectrum of psoriasis-associated pustular phenotypes that are caused by *IL36RN* alleles. To achieve this objective, we analyzed an extended collection of GPP cases and, in parallel, investigated the possibility that *IL36RN* may contribute to palmarplantar pustulosis (PPP) and acrodermatitis continua of hallopeau (ACH), two acral forms of pustular psoriasis that have been historically grouped with GPP (Griffiths and Barker, 2010), and which are also thought to be genetically distinct from PV (Asumalahti *et al.*, 2003).

We sequenced the four *IL36RN* coding exons in 84 GPP, 9 ACH, and 139 PPP cases (Supplementary Table S1 online). Owing to the rarity of the examined diseases, we defined as potentially pathogenic any non-synonymous substitution, frameshift mutation, or splicing defect that had a minor allele frequency (MAF)<0.01 in the relevant ethnic group. We observed homozygous/compound heterozygous alleles meeting the above criteria in 7/84 GPP patients, thus validating previous observations of genetic heterogeneity in this disease (Onoufriadis *et al.*, 2011; Li *et al.*, 2012; Sugiura *et al.*, 2012). Importantly, we also identified recessive *IL36RN* variants in 2/9 ACH and 3/139 PPP patients (Table 1).

The most prevalent allele in the European population was the previously characterized p.Ser113Leu substitution (Onoufriadis *et al.*, 2011), which was found in all three patient groups. The most frequent change in the Asian data set was the c.115+6T>C variant, which is known to disrupt the splicing of exon 3, leading to the synthesis of a truncated protein (Farooq *et al.*, 2012). As our resource did not include Asian PPP or ACH patients, we could not establish whether the c.115+6T>C allele also contributes to these conditions.

An analysis of intragenic SNP haplotypes indicated that both p.Ser113Leu and c.115+6T>C are likely to have

Abbreviations: ACH, acrodermatitis continua of hallopeau; FMF, familial Mediterranean fever; GPP, generalized pustular psoriasis; MAF, minor allele frequency; PPP, palmarplantar pustulosis; PV, psoriasis vulgaris